Host genotype and co-infection modify the relationship of within and between host transmission

Host genotype and co-infection modify the relationship of within and between host transmission

Hanna Susi[1], Pedro F. Vale[2,3] & Anna-Liisa Laine[1]*

[1]Metapopulation Research Group, Department of Biosciences, PO Box 65 (Viikinkaari 1), FI-00014 University of Helsinki, Finland, Email: hanna.susi@helsinki.fi, anna-liisa.laine@helsinki.fi

[2]Centre for Immunity, Infection, and Evolution, School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom, email: pferrei2@staffmail.ed.ac.uk

[3]Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom.

Correspondence: E-mail: anna-liisa.laine@helsinki.fi

Phone: +358-2941-57750

Abstract

Variation in individual-level disease transmission is well documented, but the underlying causes of this variation are challenging to disentangle in natural epidemics. In general, within-host replication is critical in determining the extent to which infected hosts shed transmission propagules but which factors cause variation in this relationship are poorly understood. Here, using a plant host *Plantago lanceolata* and the powdery mildew fungus *Podosphaera plantaginis*, we quantify how the distinct stages of within-host spread (auto-infection), spore release, and successful transmission to new hosts (allo-infection) are influenced by host genotype, pathogen genotype and the coinfection status of the host. We find that within-host spread alone fails to predict transmission rates, as this relationship is modified by genetic variation in hosts and pathogens. Their contributions change throughout the course of the epidemic. Host genotype and coinfection had particularly pronounced effects on the dynamics of spore release from infected hosts. Confidently predicting disease spread from local levels of individual transmission therefore requires a more nuanced understanding of genotype specific infection outcomes. This knowledge is a key to a better understanding of the drivers of epidemiological dynamics and the resulting evolutionary trajectories of infectious disease.

Keywords: Disease transmission, epidemiology, host-pathogen interaction, *Plantago lanceolata*, *Podosphaera plantaginis*

Introduction

Understanding the determinants of disease emergence and spread is one of the major challenges in disease biology (Gandon et al. 2013; Lively et al. 2014; May et al. 2001; Yates et al. 2006). Like most biological invasions, pathogen spread is characterized by the movement of small numbers of individuals through a spatially heterogeneous environment (Hatcher et al. 2012; Schreiber and Lloyd-Smith 2009). Small numbers of invading individuals mean that the initial phases of pathogen epidemics are highly stochastic, making the likelihood of successful transmission greatly influenced by the host a pathogen happens to infect (Gandon et al. 2013; Hartfield and Alizon 2013; Schreiber and Lloyd-Smith 2009). From the pathogen's perspective, not all hosts are equal and some hosts contribute more than others to the development of epidemics (Lloyd-Smith et al. 2005; Paull et al. 2012). Individual host heterogeneity in disease transmission is well recognized, and has been addressed both theoretically (Bifolchi et al. 2013; Lloyd-Smith et al. 2006; Matthews and Woolhouse 2005) and empirically (Courtenay et al. 2014; Cronin et al. 2010; Kilpatrick et al. 2006; VanderWaal et al. 2014; Woolhouse et al. 1997). However, while it is clear that host heterogeneity in transmission is common, we currently know little about the genetic and environmental conditions that may lead to some hosts to contribute disproportionally more to transmission (Fellous et al. 2012; Ferrari et al. 2004; Lass et al. 2013; Paull et al. 2012; Vale et al. 2013).

One facet of pathogen transmission that is currently poorly studied is the relationship between within-host colonization and between-host transmission, particularly how this relationship varies among individual hosts (Mideo et al. 2008). Once a pathogen has successfully infected a host, the process of within-host replication is expected to be critical for how much that infected host sheds transmission propagules that potentially leads to new infections. This link is a general feature of all host-parasite systems (Anderson and May 1982), regardless of the idiosyncrasies affecting exactly how

within-host infection progresses and how transmission is achieved (Bowen and Walker 2005; Ebert and Weisser 1997; Hughes et al. 2011; Quinn et al. 2000). In plant-pathogen epidemics, infection dynamics can be divided into two distinct phases. During auto-infection the pathogen spreads from the focal infection to the surrounding leaves within the same host plant. Variation in auto-infection leads to plants with varying numbers of infected leaves, and therefore auto-infection may also be seen as a measure of infection severity. During and following local spread within a host plant, allo-infection occurs, where the pathogen is transmitted to other hosts (Mundt 2009; Robinson 1976). A potentially important distinction between the auto- and allo-infection processes we describe here is that auto-infection involves the same pathogen transmission stage as allo-infection, while in most animal infections, they result from two different infection stages (for example in malaria merozoites spread infection within hosts and gametocytes transmit infection to other hosts; Bannister and Mitchell 2003; Schmidt-Hempel 2011).

Given that the window of time for a pathogen epidemic to develop is usually limited to certain environmental conditions (temperature, humidity) and host availability (Garrett et al. 2009), the optimal timing of auto- and allo-infection is crucial to the fate of the pathogen. Most epidemiological studies follow the rate of allo-infections, usually referred to as the transmission rate. Rates of auto-infection have typically received less attention, but some work has shown that host auto-infection can also vary (Lannou et al. 2008), with consequences for pathogen life history and virulence evolution (van den Berg et al. 2013). Measuring auto-infection is generally easier than quantifying the extent of allo-infection, and traditionally within-host pathogen replication is assumed to equate with transmission potential (Anderson and May 1982). Given that in most infections the symptoms arise as a consequence of the production of transmission propagules during auto-infection, we might expect hosts with severe disease symptoms to produce higher levels of transmission propagules (auto-infection), and ultimately

cause more disease transmission (during allo-infection; Beldomenico and Begon 2010). However, there are many reasons why this relationship may not always hold, and understanding them would help pinpoint which diseased individuals should be targeted in order to maximize the efficacy of disease control.

To date, the effect of host and pathogen genetic diversity on the spread of infectious disease has received little experimental exploration, and is currently one of the most pressing questions in disease ecology and evolution (Lively et al. 2014). Given that genetic variation (both of hosts and the pathogens that infect them) is well-known to affect all aspects of infection outcomes (Bergelson et al. 2001; Tack et al. 2012; Wolinska and King 2009), the relationship between auto- and allo-infection is also likely vary depending on the genetic composition both hosts and pathogens. First, pathogen populations are highly variable in their ability to infect and cause disease (Tack et al. 2012), and trade-offs between the different life-history stages of infection may also vary with genotype and genotype-by-genotype interactions (Laine and Barrès 2013). Hence, there may be considerable variation among strains in their ability to grow within an infected host, and transmit to new ones. Second, variation among hosts in how they resist becoming infected and mitigate infection development – as well as possible trade-offs between these traits - may further generate variation in auto-allo-infection dynamics (Bergelson and Purrington 1996; Susi and Laine 2015). Finally, hosts are often found to harbor mixed infections where the infection outcome cannot be predicted from those of single infections (Pedersen and Fenton 2007; Petney and Andrews 1998). Recognizing the variable nature of pathogen infections is important because mixed-genotype infections often result in increased parasite competition and virulence (Alizon et al. 2013; Choisy and de Roode 2010). Variation in the level of co-infection among individual plants is therefore likely to affect the extent of pathogen transmission propagule production during auto-infection, and influence between-host transmission. Therefore, in addition to commonly

110 studied genetic variation in host defences (Ayres and Schneider 2012; Laine et al. 2011; Råberg et al.
111 2009; Read et al. 2008; Roy and Kirchner 2000; Simms and Triplett 1994), the level of co-infection
112 may also generate individual host heterogeneity in infectiousness (Lass et al. 2013), potentially
113 modifying the dynamics of infectious disease (Streicker et al. 2013; Susi et al. 2014).

114 A clear understanding of what generates variation in the relationship between auto- and
115 allo-infection calls for controlled experiments with two major features: 1) the level of auto-infection
116 can be monitored in a non-destructive manner throughout the course of an infection across a large
117 numbers of individual hosts, and 2) the resulting pathogen propagule shedding and spreading can be
118 precisely measured for each individual host. In addition, a realistic understanding of auto- and allo-
119 infection requires studying both single and mixed infections, as these are the conditions hosts will
120 commonly experience in the wild. Fulfilling all these requirements is challenging, and so experiments
121 such as the one we describe are understandably rare.

122 Here, we take a common garden approach using a plant-pathogen system to test how host
123 genotype, pathogen genotype, coinfection and time affect 1) the level of auto-infection within the host,
124 and 2) the relationship between auto-infection and allo-infection. The experimental work was carried
125 out with powdery mildew, *Podosphaera plantaginis* infecting the host plant *Plantago lanceolata.* Our
126 experiment used multiple host genotypes that were cloned into replicates, and inoculated with two
127 pathogen strains, either singly or as a coinfection. This host-pathogen interaction is highly amenable to
128 ecological studies, as infection is visually conspicuous on host surface and the disease cycle lacks
129 extended latency periods. Hence, auto-infection can be visually directly quantified. With a combination
130 of two spore trapping methods, spore traps and live susceptible leaves; we were able to disentangle the
131 relationship between the shedding of transmission propagules and the actual spread of the infection.
132 We monitored both auto- and allo-infection dynamics over the course of an epidemic to assess how this

relationship changes over time. By genotyping the resulting infections we were also able to identify the transmitted pathogen genotypes from co-infected host plants.

Material and methods

Host-pathogen interaction

Podosphaera plantaginis is a specialist powdery mildew naturally infecting *Plantago lanceolata* in the Åland archipelago, southwestern Finland. *Plantago lanceolata* is an obligate outcrossing perennial herb that reproduces both sexually and clonally via side-rosettes. The epidemiological dynamics of *Podosphaera* in its large host population network have been studied since 2001 in the Åland Islands, southwest of Finland. These studies have demonstrated that this pathogen persists as a highly dynamic metapopulation (Jousimo et al. 2014). Visible signs of infection appear in late June in those host populations in which the pathogen has successfully overwintered as resting spores. The epidemic begins from these initial disease foci as the pathogen is transmitted by wind both within and among hosts via clonally produced dispersal spores, conidia (Laine and Hanski 2006; Ovaskainen and Laine 2006). Some six to eight clonally produced generations follow one another in quick succession and as a consequence, infection spreads within (Ovaskainen and Laine 2006) and between host populations (Jousimo et al. 2014). By September weather conditions turn unfavorable to disease transmission, and the epidemic spread ceases.

In the interaction between *Plantago* and *Podosphaera* disease resistance is strain-specific with the same host genotype blocking infection by some strains of the pathogen while being susceptible to others. There is considerable variation among host individuals and populations in their degree of resistance. The obligate pathogen can only establish on susceptible hosts, and hence, variation in

156 resistance plays a fundamental role in determining disease dynamics (Jousimo et al. 2014). Local
157 pathogen populations also support considerable genetic (Tollenaere et al. 2012) and phenotypic (Susi
158 and Laine 2013) diversity. Coinfections, whereby two or more strains of *Podosphaera* simultaneously
159 infect the same host, are common in the Åland metapopulation (Tollenaere et al. 2012).

160

161 ***Spore trapping experiment***

162 To quantify the number of fungal spores released from each host plant, and the number of successfully
163 established new infections (a measure of transmission), we carried out a spore trapping experiment. We
164 used eight generally susceptible (i.e. wide range of *Podosphaera* strains including the strains used in
165 this study are able to infect and sporulate in them) *Plantago* genotypes originating from five
166 populations in Åland (IDs 4 (three plants), 511 (two plants), 1413 (one plant), 2220 (one plant), and
167 9031 (1 plant)) as focal plants. Host plants were collected as seed in August 2010 and stored in paper
168 envelopes at room temperature. Seeds were germinated by placing them in 0.8 L pots in 50:50 sand–
169 potting soil mixture in greenhouse conditions of 16 h of light and at +22 °C. Plants were cloned in the
170 greenhouse according to the protocol described in Laine (2004), producing up to 24 ramets. Eight-
171 week-old ramets were placed outside for two weeks of acclimation until the experiment was set up. We
172 used two *Podosphaera* strains originating from Åland (strain 10 from population 2821 and strain 3
173 from population 877) that were infective on all host genotypes used in this experiment. The strains
174 were purified and maintained on fresh susceptible *Plantago* leaves on Petri dishes in a growth chamber
175 with 16:8 light: dark cycle at 20 ± 2°C.

176 To determine how host genetic background and pathogen treatment (single or co-
177 infection) affect the relationship between auto-infection and allo-infection we performed a spore

178 trapping experiment under semi-natural conditions. The experiment was set up in 2013 at the Lammi
179 Biological Station (61°05’28’’N, 25°03’90’’E) where neither *Plantago* nor *Podosphaera* occur
180 naturally; hence environmental contamination by fungal spores was highly unlikely. In mid-July the
181 experimental plants were potted in 11 cm × 11 cm pots placed at a one meter radius from each other
182 and inoculated with strain 3, strain 10 or co-inoculation of strains 3 and 10. The amount of inoculum
183 (all spores brushed off from a 1 cm^2 ten-day old sporulating lesion onto one leaf of the receiving plant)
184 was the same for all plants, with the coinfected plants receiving half of the dose of the single genotype
185 inoculum (i.e. all spores brushed from a 0.5 cm^2 lesion of strain 3 and 0.5 cm^2 lesion of strain 10 on to
186 one leaf of receiving plant). We also included a control treatment with no pathogen spores to ensure
187 there was no contamination between the plants. Four replicates of each plant genotype × pathogen
188 treatment were used and two replicates of each plant genotype and control treatment were used,
189 resulting in 112 plants in total. The auto-infection rate of the infected plants was measured as the
190 number of leaves on a plant that were infected with powdery mildew.

191 To quantify spore release and allo-infection we conducted five spore trapping sessions at
192 20, 30, 40, 50 and 60 DPI. Our study was focused on quantifying short-distance transmission at 5 cm
193 distance from the infected host, as this is a relevant distance in the high density populations of *Plantago*
194 in Åland (Laine 2004), with most spores landing very close to the infection source (< 10 cm; Tack et al.
195 2014). We used two types of traps: petroleum jelly coated microscope slides (to quantify the number of
196 spores released) and live detached *Plantago* leaves (to measure the number of spores that landed on and
197 later germinated on a host, a measure of transmission potential). Four petroleum jelly coated
198 microscope slides were attached on wooden sticks at 5 cm distance from ground placed between the
199 infected leaves in radial design to quantify spore release. Sixteen detached live leaves of known
200 susceptible genotypes were attached to moist floral foam at 5 cm distance from the focal plant to

quantify allo-infection. These trap leaves were from five generally susceptible genotypes used in strain maintenance in the laboratory. The trapping period lasted 24 hours, after which the traps were removed. The petroleum jelly traps were then kept in 5°C and subsequently examined under a microscope using four transect lines to count the released spores. The live leaves were placed on moist filter paper in a Petri dish and kept in a growth chamber. After 14 days their infection status was monitored and the infected leaves were collected for subsequent genotyping. The level of auto-infection was assessed during each trapping session by counting the number of infected leaves. All control plants remained uninfected throughout the experiment. The infection status of the plants was monitored at 20 DPI on the leaf that had received the inoculation treatment. In total 75 plants (79.2%) become infected. Plants that did not show visible signs of infection at 20 DPI (20.8%) were excluded from the analyses. To avoid unnecessary handling of the plants during the experiment, the total number of leaves in each plant was counted at the end of the experiment.

Genetic analyses

We genotyped the trap leaves infected at time points 40-60 days post-infection (DPI) to ensure that there was no cross contamination between plants, and to determine which of the pathogen strains – or both - had successfully infected the trap leaves in the coinfection treatments. From each infected leaf, we cut the lesions, consisting of both host tissue and fungal material, into a 1.5 mL tube that was kept at -20 °C until DNA was extracted using E.Z.N.A. Plant Mini Kit (Omega Bio Tek Inc. Norcross, GA, USA). Samples were genotyped using the 27 SNP panel as in Tollenaere et al. (2012). The pathogen lines used in the experiment can be distinguished from each other as they differ at eight loci used in the

222 genotyping panel. We classified leaves as coinfected if they showed presence of two alleles in
223 polymorphic loci (Tollenaere et al. 2012).

224

225 ***Statistical analyses***

226 To test how host genotype, pathogen genotype, coinfection and time affect on 1) auto-infection, 2) how
227 auto-infection correlates with spore release, 3) how auto-infection correlates with infection
228 establishment, and 4) how spore release correlates with infection establishment we performed four
229 different analyses using Generalized Linear Mixed Models in SAS 9.2 (SAS Institute 2011) using the
230 GLIMMIX procedure (Littell et al. 2006). To determine the factors that influence auto-infection, we
231 analyzed the proportion of infected leaves as the response variable where number of infected leaves
232 was used as defined as numerator and number of all leaves in a plant as denominator, with plant
233 genotype and pathogen treatment as explanatory categorical variables; the number of leaves on a plant
234 (counted at 60 DPI) and time (DPI) were included as covariates. Plant replicate, hierarchically nested
235 under plant genotype, was defined as a random variable. The model was fitted with a binomial
236 distribution of errors. In order to evaluate the relationship between auto-infection rate and spore release
237 we analyzed the number of spores released from each plant (the number of spores in the four
238 microscope slides) as the response variable with a Poisson distribution of errors, plant genotype and
239 pathogen treatment as explanatory categorical variables, with number of infected leaves (auto-infection
240 level) and time as days post-infection (DPI) as covariates. Plant replicate nested within genotype was
241 treated as a random variable to control for possible variation due to the location of the plant in the study
242 area.

To understand the relationship between auto-infection and allo-infection we analyzed the proportion of infected trap leaves (as a numerator) of all trap leaves (as denominator) around the focal plant as a response variable with a binomial distribution of errors. There was overdispersion in the data and we chose Complementary Log-Log link function to fit the model. The level of autoinfection was included as a covariate, otherwise the model variables are the same as described above. Finally, to understand the relationship between transmission potential and infection establishment, we analyzed how spore release affects the establishment of infection by having the proportion of infected trap leaves (as numerator) of all trap leaves (denominator) as a response variable, spore release (number of spores trapped on the four microscope slides) as a covariate, and plant genotype and pathogen treatment as explanatory categorical variables, and time as DPI as a covariate. We used binomial distribution of errors and Complementary Log-Log link function. To identify differences within significant main effects we used post hoc comparisons by computing least squared means of the main effects in SAS 9.2 Proc Glimmix (Littell et al. 2006). In all analyses non-significant interactions were excluded from the final models.

Results

Factors determining auto-infection

The fraction of infected leaves increased with time and there was weak negative correlation between number of leaves in a plant and the fraction of diseased leaves (Supplementary Figure 1; Supplementary Table 1). Plant genotype and pathogen treatment had a significant effect on the fraction of the diseased leaves (Supplementary Figure 1; Supplementary Table 1). By the end of the experiment,

none of the plants were saturated with infection: 40% or fewer of the leaves were infected (Supplementary Figure 1).

The dynamics of pathogen spore release

Spore release (estimated as the number of spores landing on the petroleum jelly traps) changed throughout the experiment, and across all treatments, peaking at 50 DPI (Figure 1, Table 1). The number of spores released depended on whether the hosts were infected singly or co-infected, with co-infected hosts shedding more pathogen spores (Figure 1A, Table 1). Post hoc comparisons revealed that while the overall number of spores released in the single infections did not differ, there were significant differences between the strains at 40 and 50 DPI (40 DPI; $P < 0.0001$; 50 DPI; $P = < 0.0001$). This is especially clear at 50 DPI, where strain 3 released more spores than 10. The number of spores released from coinfected plants was highest at 50 DPI (Figure 1A). At 60 DPI the overall number of spores released decreased and the differences between treatments diminished (Figure 1A).

The amount of spores released depended also on the host genotype. Averaged across all single and coinfections, some host genotypes (G1, G3 and G6) produced more spores than others throughout the epidemic (Figure 1B, Table 1). The effect of host genetic variation on infectiousness is especially pronounced during the peak spore release at 50 DPI (Figure 1B) when the difference in spore release was the largest. Overall, the effect of pathogen treatment on spore release changed through time (significant interaction between time and treatment in Table 1; Figure 1A). The amount of spores released from coinfected plants was highest at 30 and 50 DPI, while at 40 DPI strain 10 had higher spore release than strain 3 and the coinfection treatment (Figure 1A). At the peak of epidemics strain 3

285 released more spores than strain 10 in single infections (Figure 1A). At 60 DPI the differences between
286 treatments diminished (Figure 1A).

287

288 ***The relationship between auto-infection and spore release***

289 One aim of our experiment was to explore how host genotype and coinfection status contributed to
290 variation in the relationship between auto-infection and spore release. As expected, high auto-infection
291 generally led to high levels of spore release (significant auto-infection term in Table 1). However,
292 while auto-infection and spore release are clearly correlated, the strength of this relationship changed
293 over the course of the epidemic, and was affected by both host and pathogen genotypes (Figure 2;
294 Table 1). Specifically, in hosts infected singly with strain 10, we observed a positive correlation
295 between the rates of auto-infection and spore release, while in plants infected with strain 3 this
296 correlation was still positive, but weaker (Figure. 2A-C; Table 1). In host plants coinfected with both
297 strains the relationship between auto-infection and spore release was highly variable (Figure 2A-C;
298 Table 1). Across all single infection and coinfection treatments, there is also variation in the
299 relationship that arises from different host genetic backgrounds. In some host genotypes (e.g. G1 and
300 G2) high auto-infection yielded high spore release whereas in other genotypes (e.g. G4, G5 and G6) the
301 relationship was weaker (Figure 2D-F; Table1).

302

303 ***The establishment of new infections***

304 Consistent with what we observed for spore release, the establishment of infection by spores landing on
305 trap leaves also peaked at 50 DPI (Figure 3A, Table 2). We found that high auto-infection rates led to

high rates of new infections becoming established, but we found no significant effects of host genotype and pathogen treatment (Table 2). The relationship between the number of spores that were released from the infected plant and the establishment of new infections varied according to which pathogen strain was involved (Spores × Treatment Figure 3B, Table 3). Generally, when hosts were infected with strain 10, shedding high numbers of spores led to the establishment of many new infections, while this relationship was noticeably weaker when hosts were co-infected with strains 3 and 10 (Figure 3B, Table 3). Genotyping of the infected trap leaves at 40-60 DPI from the coinfection treatment revealed that 40% were infected by strain 3, 40% by strain 10, and 20% of new infections consisted of both strains.

Discussion

Taken together, our results highlight the host's heterogeneous contribution to the temporal dynamics of epidemics. We found that the contribution of a host individual to epidemics varies over time and correlates with its level of auto-infection as expected, but importantly, also depends on the host genetic background and whether it is coinfected or singly infected. We also tested a common assumption that rapid colonization of the host by the pathogen (auto-infection) is associated with the ability to spread infection between hosts (e.g. Robinson 1976). Our results indicate that host and pathogen genetic background can mediate the temporal dynamics of within- and between-host pathogen spread.

The effect of pathogen strain on transmission dynamics

In this study we found that, after controlling for the level of auto-infection in our models, the overall spore release and infection establishment rates between the singly infecting strains did not differ.

328 However, we observed significant differences between the strains at individual time points during the
329 epidemics (Figure 1A), and importantly, differences in how many spores were released at different
330 levels of auto-infection (Figure 2). In plants infected with pathogen strain 10 there was a stronger
331 correlation than in the case of plants infected with strain 3. This may indicate a difference in the latent
332 period of the two strains: the weaker correlation for strain 3 spores may result from a longer period
333 between auto-infection and spore shedding. This suggests that different pathogen strains may have
334 different optimization strategies between within-host and between-host levels. The timing of
335 transmission is essential in the disease dynamics as the time window for infection spread is usually
336 limited by the environment, host life span or behavior (Hartfield and Alizon 2013). The differences in
337 transmission at the early stages of the epidemics may have profound consequences for disease
338 epidemiology and pathogen evolution, as rapid transmission is expected to lead to a greater share of the
339 prevailing pathogen population (Day 2003) and therefore higher pathogen fitness (Elena 2001). We
340 also found that in hosts infected with strain 10 the amount of spores released accurately predicted
341 infection establishment while in strain 3 this trend was not as clear, suggesting that there are
342 differences between the strains in their spore quality (Figure 3B).

343

344 ***Host-mediated variation in within and between host dynamics***

345 Auto-infection and allo-infection dynamics have been approached theoretically in epidemiological
346 modelling (Mideo et al. 2008; Mundt and Leonard 1986; Willocquet and Savary 2004), but empirical
347 studies remain rare and the host's role in mediating the dynamics has been largely unexplored. We
348 found heterogeneity in the contributions of different host genotypes to pathogen replication (Figure
349 1B), consistent with the idea that host genotype is critical for infection development (Salvaudon et al.

2008). Not only did host genotypes differ in the number of spores released, the relationship between spore release and infection establishment was also significantly determined by the host genetic background. One reason the relationship between auto-infection and spore release may vary is because hosts may differ in the types of defenses employed during infection, and in the level of nutrients available for the pathogen (Laine 2007). Hosts have evolved various strategies to reduce the harm caused by the pathogen (Ayres and Schneider 2012; Dodds and Rathjen 2010; Råberg et al. 2009; Read et al. 2008; Simms and Triplett 1994). One strategy is to resist the pathogen by mitigating its growth, leading to clearance and recovery (Alexander 1992; Schmidt-Hempel 2011). Another strategy is to minimize the negative fitness effects of infection through damage limitation mechanisms that allow hosts to tolerate the presence of pathogens (Ayres and Schneider 2012; Medzhitov et al. 2012; Roy and Kirchner 2000). It remains unclear how the interaction between different host defense strategies may shape the relationship between auto- and allo-infection spread. For example, hosts investing heavily in costly resistance mechanisms could clear pathogens, leading to reduced transmission, but could also experience severe symptoms due to immunopathology (Graham et al. 2005; Jones and Dangl 2006). Conversely, hosts investing heavily in damage limitation mechanisms may show only mild disease symptoms (Poland et al. 2009) but would still tolerate the production of transmission propagules during auto-infection and contribute considerably to transmission during allo-infection (Vale et al. 2014). We used host genotypes where mildew was able to establish and grow, but it is possible that host genotypes differed in their ability to tolerate infection. Infection tolerance has not been systematically explored in this system, but is an important line of future research. In general, an open question for future studies is how the relationship between auto-infection and spore release may vary between host genotypes with differential investment in resistance or tolerance strategies.

373 *The effect of coinfection on transmission dynamics*

374 We found that transmission dynamics were altered under coinfection; the overall highest spore
375 shedding was observed in coinfected plants (Figure 1A). This result is in line with a study on mice
376 where coinfection lead to higher helminth oviposition (Lass et al. 2013). Interestingly, in the early
377 phase of the epidemic at 40 DPI, auto-infection rate was not a strong predictor of spore release in the
378 coinfected plants – high spore release was observed at low auto-infection levels while host plants with
379 high levels of auto-infection released a low number of spores (Figure 2). As the epidemic progressed, a
380 positive correlation between auto-infection and spore release emerged. It has also been suggested
381 previously that the relationship between host disease severity and pathogen transmission may be altered
382 in co-infection (Bremermann and Pickering 1983). Here, we found that the correlation between spore
383 release and allo-infection was weaker under coinfection than in the singly infecting strains. This
384 finding suggests that the accelerated spore production we observe under coinfection comes at a cost of
385 spore quality. These effects of co-infection are likely to be relevant more broadly, as hosts are
386 frequently infected by more than a single pathogen strain (Balmer and Tanner 2011; Lopez-
387 Villavicencio et al. 2007; Telfer et al. 2010) and, in some cases it has been shown that coinfection may
388 lead to increased reproduction of the pathogen (Lass et al. 2013).

389 Hosts are bottlenecks of pathogen genetic diversity and can therefore shape pathogen epidemics
390 (Fellous et al. 2012; Tack et al. 2014) and evolution (Cisarovsky and Schmid-Hempel 2014). High
391 levels of mixed infections can therefore maintain pathogen genetic diversity at the level of individual
392 hosts, but it is poorly understood if this diversity spreads to other hosts (Lively et al. 2014). Our
393 genotyping revealed that 20% of the infections resulting from coinfected plants were also coinfected
394 suggesting the possibility of co-transmission, while the single infections established at equal
395 probability from the coinfected source (both strains found singly in 40% of the infected leaf traps).

Whether pathogens spread as single strains or are co-transmitted may have consequences for epidemiology as infection success has been reported to increase in co-transmission (Karvonen et al. 2012) and is assumed to have further consequences for the evolution of virulence (Alizon 2013). In the case of vector transmitted pathogens, simultaneous transmission of different strains and species is well documented (Pirone and Blanc 1996) but our study is among the few to investigate the frequency of co-transmission in airborne pathogens. Applied to natural populations, where multihost-multi-pathogen interaction networks are the norm (Pedersen and Fenton 2007), our results lend insight into a currently outstanding question in disease ecology and evolution (Lively et al. 2014): how pathogen interactions within hosts may regulate pathogen diversity at the population level.

Limitations of the current study

In this study we found that the relationship between spore release and infection establishment was mediated only by pathogen genetic background, but we detected no effect of the focal host genotype (Figure 3). However, it is important to emphasize that the overall proportion of infected trap leaves was low, 0.014. While this is comparable to the estimated success of single bouts of transmission (Ovaskainen and Laine 2006), it may have reduced our power to detect the effects of these potential sources of variation. The size of the current experimental design was determined by the need to study spore release, auto-infection and allo-infection. In order to increase statistical power to fully disentangle the sources of variation on successful disease spread during allo-infection, future studies should focus on the realized transmission on large numbers of host genotypes representing different resistance backgrounds and large numbers of pathogen stains with different infection profiles. Moreover, as previous studies have found evidence of pathogen local adaption to host resistance (Laine

418 2005; Laine 2008), it would be highly relevant to determine how coevolutionary dynamics may alter
419 the relationship between auto- and allo-infection.

420

421 ***Conclusions***

422 We detected important effects of host genotype and co-infection status on the temporal dynamics of
423 spore release, which re-enforces the need to identify co-infection in wild-hosts as a potential risk factor
424 of disease spread (Mideo et al. 2008; Susi et al. 2014). Our results also suggest that the degree to which
425 a host plant sheds fungal spores onto its own leaves is not necessarily a good predictor of spread to
426 neighboring host plants: we found the correlation between autoinfection and allo-infection to change
427 over the course of our experimental epidemic, and to vary particularly in strength according to the host
428 co-infection status. This result has important implications for the control of disease. In trying to delay
429 the spread of infection, it would seem logical to remove or treat hosts showing the clearest signs of
430 disease. While this may be true generally, our results suggest that timing is crucial, as a strong positive
431 relationship between auto-infection and spore release only became apparent as the epidemic
432 progressed. When designing disease management efforts, it is therefore important to understand the
433 relationship between auto-infection, spore shedding and allo-infection, and how the relationship
434 between them may vary according to the genetic and environmental context experienced by hosts.

435

436 **Acknowledgements**

437 Lammi Biological Station is acknowledged for hosting the experimental study, and P. Hyttinen and S.
438 Hartikainen are acknowledged for their help in the field. L. Ruokolainen is acknowledged for statistical

advice. N. Mideo and R. Penczykowski are acknowledged for helpful comments on the earlier version of the manuscript. B. Barrès helped analyze the genetic samples and Institute of Biotechnology and Institute for Molecular Medicine at the University of Helsinki are acknowledged for carrying out the DNA extractions and genotyping of samples, respectively. HS is funded by LUOVA Doctoral School in Wildlife Biology Research and PFV is funded by a strategic award from the Wellcome Trust for the Centre for Immunity, Infection and Evolution (http://ciie.bio.ed.ac.uk; grant reference no. 095831), and by a Scottish Universities Life Sciences Alliance (SULSA) LEADERS mobility award. This work was supported by funding from the Academy of Finland (Grant Nos 250444, 136393, 133499) and the European Research Council (Independent Starting Grant PATHEVOL; 281517) to A.-L.L.

References

Alexander, H. M. 1992. Evolution of disease resistance in natural plant populations, Pages 326-344 *in* R. S. Fritz, and E. L. Simms, eds. Plant Resistance to Herbivores and Pathogens Ecology, Evolution and Genetics. Chicago, The University of Chicago Press.

Alizon, S. 2013. Parasite co-transmission and the evolutionary epidemiology of virulence. Evolution 67:921-933.

Alizon, S., J. C. de Roode, and Y. Michalakis. 2013. Multiple infections and the evolution of virulence. Ecology Letters 16:556-567.

Anderson, R. M., and R. M. May. 1982. Coevolution of hosts and parasites. Parasitology 85:411-426.

460 Ayres, J. S., and D. S. Schneider. 2012. Tolerance of Infections. Annual Review of Immunology, Vol
461 30 30:271-294.
462 Balmer, O., and M. Tanner. 2011. Prevalence and implications of multiple-strain infections. Lancet
463 Infectious Diseases 11:868–887.
464 Bannister, L., and G. Mitchell. 2003. The ins, outs and roundabouts of malaria. Trends in Parasitology
465 19:209-213.
466 Beldomenico, P. M., and M. Begon. 2010. Disease spread, susceptibility and infection intensity:
467 vicious circles? Trends in Ecology & Evolution 25:21-27.
468 Bergelson, J., M. Kreitman, E. A. Stahl, and D. C. Tian. 2001. Evolutionary dynamics of plant R-
469 genes. Science 292:2281-2285.
470 Bergelson, J., and C. B. Purrington. 1996. Surveying patterns in the cost of resistance in plants.
471 American Naturalist 148:536-558.
472 Bifolchi, N., R. Deardon, and Z. Feng. 2013. Spatial approximations of network-based individual level
473 infectious disease models. Spatial and Spatio-temporal Epidemiology 6:59-70.
474 Bowen, D. G., and C. M. Walker. 2005. Adaptive immune responses in acute and chronic hepatitis C
475 virus infection. Nature 436:946-952.
476 Bremermann, H. J., and J. Pickering. 1983. A game-theoretical model of parasite virulence. Journal of
477 Theoretical Biology 100:411-426.
478 Choisy, M., and J. C. de Roode. 2010. Mixed infections and the evolution of virulence: effects of
479 resource competition, parasite plasticity, and impaired host immunity. American Naturalist
480 175:E105-118.
481 Cisarovsky, G., and P. Schmid-Hempel. 2014. Few colonies of the host *Bombus terrestris*
482 disproportionately affect the genetic diversity of its parasite, C*rithidia bombi*. Infection,
483 Genetics and Evolution 21:192-197.

484 Courtenay, O., C. Carson, L. Calvo-Bado, L. M. Garcez, and R. J. Quinnell. 2014. Heterogeneities in
485 *Leishmania infantum* infection: using skin parasite burdens to identify highly infectious dogs.
486 PLoS Neglected Tropical Diseases 8:26.
487 Cronin, J. P., M. E. Welsh, M. G. Dekkers, S. T. Abercrombie, and C. E. Mitchell. 2010. Host
488 physiological phenotype explains pathogen reservoir potential. Ecology Letters 13:1221-1232.
489 Day, T. 2003. Virulence evolution and the timing of disease life-history events. Trends in Ecology &
490 Evolution 18:113-118.
491 Dodds, P. N., and J. P. Rathjen. 2010. Plant immunity: towards an integrated view of plant-pathogen
492 interactions. Nature Reviews Genetics 11:539-548.
493 Ebert, D., and W. W. Weisser. 1997. Optimal killing for obligate killers: The evolution of life histories
494 and virulence of semelparous parasites. Proceedings of the Royal Society B-Biological Sciences
495 264:985-991.
496 Elena, S. F. 2001. Evolutionary history conditions the timing of transmission in vesicular stomatitis
497 virus. Infection, Genetics and Evolution 1:151-159.
498 Fellous, S., A. B. Duncan, E. Quillery, P. F. Vale, and O. Kaltz. 2012. Genetic influence on disease
499 spread following arrival of infected carriers. Ecology Letters 15:186-192.
500 Ferrari, N., I. M. Cattadori, J. Nespereira, A. Rizzoli, and P. J. Hudson. 2004. The role of host sex in
501 parasite dynamics: field experiments on the yellow-necked mouse *Apodemus flavicollis*.
502 Ecology Letters 7:88-94.
503 Gandon, S., M. E. Hochberg, R. D. Holt, and T. Day. 2013. What limits the evolutionary emergence of
504 pathogens? Philosophical Transactions of the Royal Society B-Biological Sciences 368.
505 Garrett, K. A., L. N. Zuniga, E. Roncal, G. A. Forbes, C. C. Mundt, Z. Su, and R. J. Nelson. 2009.
506 Intraspecific functional diversity in hosts and its effect on disease risk across a climatic
507 gradient. Ecological Applications 19:1868-1883.

508 Graham, A. L., J. E. Allen, and A. F. Read. 2005. Evolutionary causes and consequences of
509 immunopathology. Annual Review of Ecology Evolution and Systematics 36:373-397.
510 Hartfield, M., and S. Alizon. 2013. Introducing the outbreak threshold in epidemiology. Plos Pathogens
511 9:e1003277.
512 Hatcher, M. J., J. T. A. Dick, and A. M. Dunn. 2012. Disease emergence and invasions. Functional
513 Ecology 26:1275-1287.
514 Hughes, D. P., S. B. Andersen, N. L. Hywel-Jones, W. Himaman, J. Billen, and J. J. Boomsma. 2011.
515 Behavioral mechanisms and morphological symptoms of zombie ants dying from fungal
516 infection. BMC Ecology 11:13.
517 Jones, J. D. G., and J. L. Dangl. 2006. The plant immune system. Nature 444:323-329.
518 Jousimo, J., A. J. M. Tack, O. Ovaskainen, T. Mononen, H. Susi, C. Tollenaere, and A. L. Laine. 2014.
519 Ecological and evolutionary effects of fragmentation on infectious disease dynamics. Science
520 344:1289-1293.
521 Karvonen, A., C. Rellstab, K.-R. Louhi, and J. Jokela. 2012. Synchronous attack is advantageous:
522 mixed genotype infections lead to higher infection success in trematode parasites. Proceedings
523 of the Royal Society B-Biological Sciences 279:171-176.
524 Kilpatrick, A. M., P. Daszak, M. J. Jones, P. P. Marra, and L. D. Kramer. 2006. Host heterogeneity
525 dominates West Nile virus transmission. Proceedings of the Royal Society B-Biological
526 Sciences 273:2327-2333.
527 Laine, A. L. 2004. Resistance variation within and among host populations in a plant-pathogen
528 metapopulation: implications for regional pathogen dynamics. Journal of Ecology 92:990-1000.
529 —. 2005. Spatial scale of local adaptation in a plant-pathogen metapopulation. Journal of Evolutionary
530 Biology 18:930-938.

—. 2007. Pathogen fitness components and genotypes differ in their sensitivity to nutrient and temperature variation in a wild plant-pathogen association. Journal of Evolutionary Biology 20:2371-2378.

—. 2008. Temperature-mediated patterns of local adaptation in a natural plant-pathogen metapopulation. Ecology Letters 11:327-337.

Laine, A. L., and B. Barrès. 2013. Epidemiological and evolutionary consequences of life-history trade-offs in pathogens. Plant Pathology 62:96-105.

Laine, A. L., J. J. Burdon, P. N. Dodds, and P. H. Thrall. 2011. Spatial variation in disease resistance: from molecules to metapopulations. Journal of Ecology 99:96-112.

Laine, A. L., and I. Hanski. 2006. Large-scale spatial dynamics of a specialist plant pathogen in a fragmented landscape. Journal of Ecology 94:217-226.

Lannou, C., S. Soubeyrand, L. Frezal, and J. Chadœuf. 2008. Autoinfection in wheat leaf rust epidemics. New Phytologist 177:1001-1011.

Lass, S., P. J. Hudson, J. Thakar, J. Saric, E. Harvill, R. Albert, and S. E. Perkins. 2013. Generating super-shedders: co-infection increases bacterial load and egg production of a gastrointestinal helminth. J R Soc Interface 10:20120588.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006, SAS for Mixed Models. Cary NC, SAS Institute Inc.

Lively, C. M., J. C. De Roode, M. A. Duffy, A. L. Graham, and B. Koskella. 2014. Interesting open questions in disease ecology and evolution. The American Naturalist 184:S1-S8.

Lloyd-Smith, J. O., S. J. Schreiber, and W. M. Getz. 2006. Moving beyond averages: Individual-level variation in disease transmission. Mathematical Studies on Human Disease Dynamics: Emerging Paradigms and Challenges 410:235-258.

554 Lloyd-Smith, J. O., S. J. Schreiber, P. E. Kopp, and W. M. Getz. 2005. Superspreading and the effect of
555 individual variation on disease emergence. Nature 438:355-359.
556 Lopez-Villavicencio, M., O. Jonot, A. Coantic, M. E. Hood, J. Enjalbert, and T. Giraud. 2007. Multiple
557 infections by the anther smut pathogen are frequent and involve related strains, Pages e176,
558 PLoS Pathogens.
559 Matthews, L., and M. Woolhouse. 2005. New approaches to quantifying the spread of infection. Nature
560 Reviews Microbiology 3:529-536.
561 May, R. M., S. Gupta, and A. R. McLean. 2001. Infectious disease dynamics: what characterizes a
562 successful invader? Philosophical Transactions of the Royal Society of London Series B-
563 Biological Sciences 356:901-910.
564 Medzhitov, R., D. S. Schneider, and M. P. Soares. 2012. Disease tolerance as a defense strategy.
565 Science 335:936-941.
566 Mideo, N., S. Alizon, and T. Day. 2008. Linking within- and between-host dynamics in the
567 evolutionary epidemiology of infectious diseases. Trends in Ecology & Evolution 23:511-517.
568 Mundt, C. C. 2009. Importance of autoinfection to the epidemiology of polycyclic foliar disease.
569 Phytopathology 99:1116-1120.
570 Mundt, C. C., and K. J. Leonard. 1986. Analysis of factors affecting disease increase and spread in
571 mixtures of immune and susceptible plants in computer-simulated epidemics. Phytopathology
572 76:832-840.
573 Ovaskainen, O., and A. L. Laine. 2006. Inferring evolutionary signals from ecological data in a plant-
574 pathogen metapopulation. Ecology 87:880-891.
575 Paull, S. H., S. Song, K. M. McClure, L. C. Sackett, A. M. Kilpatrick, and P. T. J. Johnson. 2012. From
576 superspreaders to disease hotspots: linking transmission across hosts and space. Frontiers in
577 Ecology and the Environment 10:75-82.

578 Pedersen, A. B., and A. Fenton. 2007. Emphasizing the ecology in parasite community ecology. Trends
579 in Ecology & Evolution 22:133-139.
580 Petney, T. N., and R. H. Andrews. 1998. Multiparasite communities in animals and humans: frequency,
581 structure and pathogenic significance. International Journal for Parasitology 28:377-393.
582 Pirone, T. P., and S. Blanc. 1996. Helper-dependent vector transmission of plant viruses. Annual
583 Review of Phytopathology 34:227-247.
584 Poland, J. A., P. J. Balint-Kurti, R. J. Wisser, R. C. Pratt, and R. J. Nelson. 2009. Shades of gray: the
585 world of quantitative disease resistance. Trends in Plant Science 14:21-29.
586 Quinn, T. C., M. J. Wawer, N. Sewankambo, D. Serwadda, C. J. Li, F. Wabwire-Mangen, M. O.
587 Meehan et al. 2000. Viral load and heterosexual transmission of human immunodeficiency virus
588 type 1. New England Journal of Medicine 342:921-929.
589 Råberg, L., A. L. Graham, and A. F. Read. 2009. Decomposing health: tolerance and resistance to
590 parasites in animals. Philosophical Transactions of the Royal Society B-Biological Sciences
591 364:37-49.
592 Read, A. F., A. L. Graham, and L. Råberg. 2008. Animal defenses against infectious agents: is damage
593 control more important than pathogen control? Plos Biology 6:2638-2641.
594 Robinson, R. R. 1976, Plant Pathosystems: Advanced Series in Agricultural Sciences, v. 3. Berlin,
595 Springer Verlag.
596 Roy, B. A., and J. W. Kirchner. 2000. Evolutionary dynamics of pathogen resistance and tolerance.
597 Evolution 54:51-63.
598 Salvaudon, L., T. Giraud, and J. A. Shykoff. 2008. Genetic diversity in natural populations: a
599 fundamental component of plant–microbe interactions. Current Opinion in Plant Biology
600 11:135-143.
601 SAS Institute. 2011.The SAS system for Windows. Release 9.2. .SAS Institute Inc., Cary, NC.

602 Schmidt-Hempel, P. 2011, Evolutionary parasitology: the integrated study of infections, immunology,
603 ecology, and genetics. Oxford, Oxford university Press.
604 Schreiber, S. J., and J. O. Lloyd-Smith. 2009. Invasion dynamics in spatially heterogeneous
605 environments. American Naturalist 174:490-505.
606 Simms, E. L., and J. Triplett. 1994. Costs and benefits of plant-responses to disease - resistance and
607 tolerance. Evolution 48:1973-1985.
608 Streicker, D. G., A. Fenton, and A. B. Pedersen. 2013. Differential sources of host species
609 heterogeneity influence the transmission and control of multihost parasites. Ecology Letters
610 16:975-984.
611 Susi, H., B. Barres, P. F. Vale, and A. L. Laine. 2014. Coinfection alters population dynamics of
612 infectious disease. Submitted.
613 Susi, H., and A.-L. Laine. 2015. The effectiveness and costs of pathogen resistance strategies in a
614 perennial plant. Journal of Ecology:Early View.
615 Susi, H., and A. L. Laine. 2013. Pathogen life-history trade-offs revealed in allopatry. Evolution
616 67:3362-3370.
617 Tack, A. J. M., J. Hakala, T. Petäjä, M. Kulmala, and A.-L. Laine. 2014. Genotype and spatial structure
618 shape pathogen dispersal and disease dynamics at small spatial scales. Ecology 95:703-714.
619 Tack, A. J. M., P. H. Thrall, L. G. Barrett, J. J. Burdon, and A. L. Laine. 2012. Variation in infectivity
620 and aggressiveness in space and time in wild host-pathogen systems: causes and consequences.
621 Journal of Evolutionary Biology 25:1918-1936.
622 Telfer, S., X. Lambin, R. Birtles, P. Beldomenico, S. Burthe, S. Paterson, and M. Begon. 2010. Species
623 interactions in a parasite community drive infection risk in a wildlife population. Science
624 330:243-246.

625 Tollenaere, C., H. Susi, J. Nokso-Koivisto, P. Koskinen, A. Tack, P. Auvinen, L. Paulin et al. 2012.
626 SNP Design from 454 sequencing of *Podosphaera plantaginis* transcriptome reveals a
627 genetically diverse pathogen metapopulation with high levels of mixed-genotype infection,
628 Pages e52492, Plos One.
629 Vale, P., A. Fenton, and S. Brown. 2014. Limiting damage during Infection: lessons from infection
630 tolerance for novel therapeutics, Pages e1001769, PLoS Biology
631 Vale, P. F., M. Choisy, and T. J. Little. 2013. Host nutrition alters the variance in parasite transmission
632 potential. Biology Letters 9:20121145.
633 van den Berg, F., S. Gaucel, C. Lannou, C. A. Gilligan, and F. van den Bosch. 2013. High levels of
634 auto-infection in plant pathogens favour short latent periods: a theoretical approach.
635 Evolutionary Ecology 27:409-428.
636 VanderWaal, K. L., E. R. Atwill, L. A. Isbell, and B. McCowan. 2014. Linking social and pathogen
637 transmission networks using microbial genetics in giraffe (*Giraffa camelopardalis*). Journal of
638 Animal Ecology 83:406-414.
639 Willocquet, L., and S. Savary. 2004. Modeling plant disease epidemics in a spatial hierarchy.
640 Phytopathology 94:S110-S110.
641 Wolinska, J., and K. C. King. 2009. Environment can alter selection in host-parasite interactions.
642 Trends in Parasitology 25:236-244.
643 Woolhouse, M. E. J., C. Dye, J. F. Etard, T. Smith, J. D. Charlwood, G. P. Garnett, P. Hagan et al.
644 1997. Heterogeneities in the transmission of infectious agents: Implications for the design of
645 control programs. Proceedings of the National Academy of Sciences of the United States of
646 America 94:338-342.

Yates, A., R. Antia, and R. R. Regoes. 2006. How do pathogen evolution and host heterogeneity interact in disease emergence? Proceedings of the Royal Society B-Biological Sciences 273:3075-3083.

Figure legends

Figure 1. Variation in spore release (number of trapped spores; solid lines) and auto-infection (number of infected leaves; dashed line) of *Podosphaera plantaginis* throughout the experiment. Coinfected plants released more spores than singly infected plants (A). Spore release varied among the eight *Plantago lanceolata* host genotypes and through time (B). Standard error of the mean is shown.

Figure 2. The relationship between auto-infection and allo-infection of *Podosphaera plantaginis.* Relationship between proportion of infected leaves in the focal plant and the spore release of *Podosphaera* strains 3 and 10 singly and under coinfection (A-C). The relationship between the proportion of infected leaves and spore release on different *Plantago lanceolata* genotypes across all pathogen treatments (D-F).

Figure 3. Infection establishment on the live leaf traps varied through time (A). The relationship between spore release and infection establishment varied according to pathogen treatment (B). Standard error of the mean is shown.

668 **Tables**

669 **Table 1.** Sources of variation in *Podosphaera plantaginis* spore release analyzed with a Generalized
670 linear mixed model. Statistically significant ($P < 0.05$) results are shown in bold.

671

Effect	**d.f.**	***F***	***P***
Time	1,326	822.16	**< 0.0001**
Treatment	2,326	16.33	**< 0.0001**
Autoinfection	1,326	689.14	**0.0006**
Genotype	7,24	3.6	**0.0086**
Time × treatment	2,326	42.07	**< 0.0001**
Autoinfection × treatment	2,326	45.2	**< 0.0001**
Autoinfection × genotype	7,326	50.3	**< 0.0001**
Autoinfection × time	1,326	793.27	**< 0.0001**

672

673

Table 2. Sources of variation in *Podosphaera plantaginis* infection establishment on trap leaves analyzed with a Generalized linear mixed model. Statistically significant ($P < 0.05$) results are shown in bold. Akaike information criterion (AIC) value of the model was 433.45.

Effect	**d.f.**	***F***	***P***
Time	1,337	1.03	0.31
Treatment	2,337	0.36	0.6997
Autoinfection	1,337	22.56	**< 0.0001**
Genotype	7,337	0.69	0.6765
Autoinfection × time	1,337	17.03	**< 0.0001**

692

693 **Table 3.** The relationship between spore release and infection establishment in *Podosphaera*

694 *plantaginis*. Results of spore trapping experiment analyzed with a Generalized linear mixed model.

695 Statistically significant ($P < 0.05$) results are shown in bold. AIC value of the model was 441.19.

696

Effect	**d.f.**	***F***	***P***
Time	1,335	0	0.9475
Treatment	2,335	3.44	**0.0331**
Spores	1,335	19.29	**< 0.0001**
Genotype	7,24	0.41	0.8836
Treatment × spores	2,335	4.18	**0.0161**

697

698

699

700

701

702

703

704

705

706

707

708

709

Figure 1.

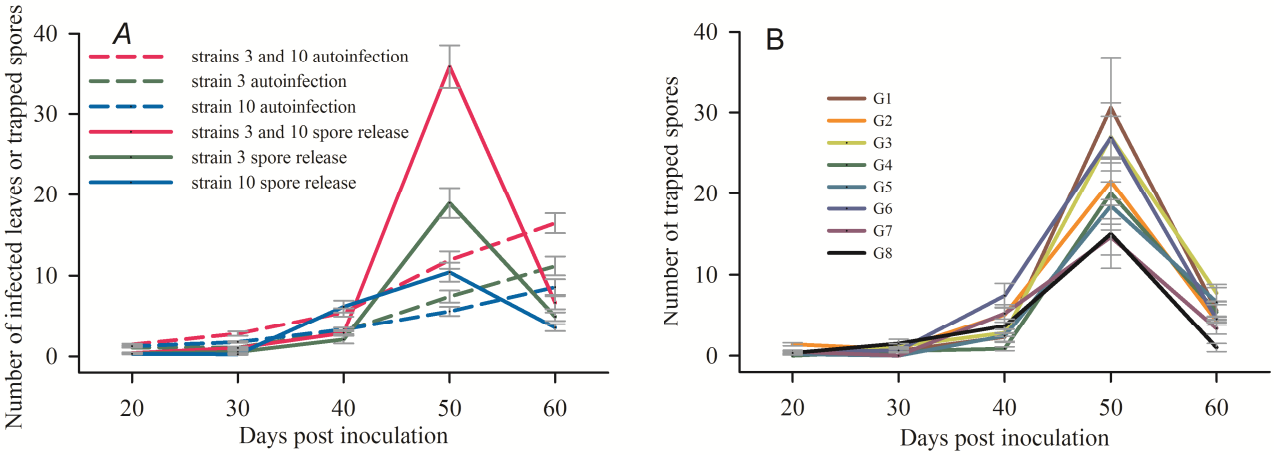

A
strains 3 and 10 autoinfection
strain 3 autoinfection
strain 10 autoinfection
strains 3 and 10 spore release
strain 3 spore release
strain 10 spore release
Number of infected leaves or trapped spores
Days post inoculation
B
G1
G2
G3
G4
G5
G6
G7
G8
Number of trapped spores
Days post inoculation

Figure 2.

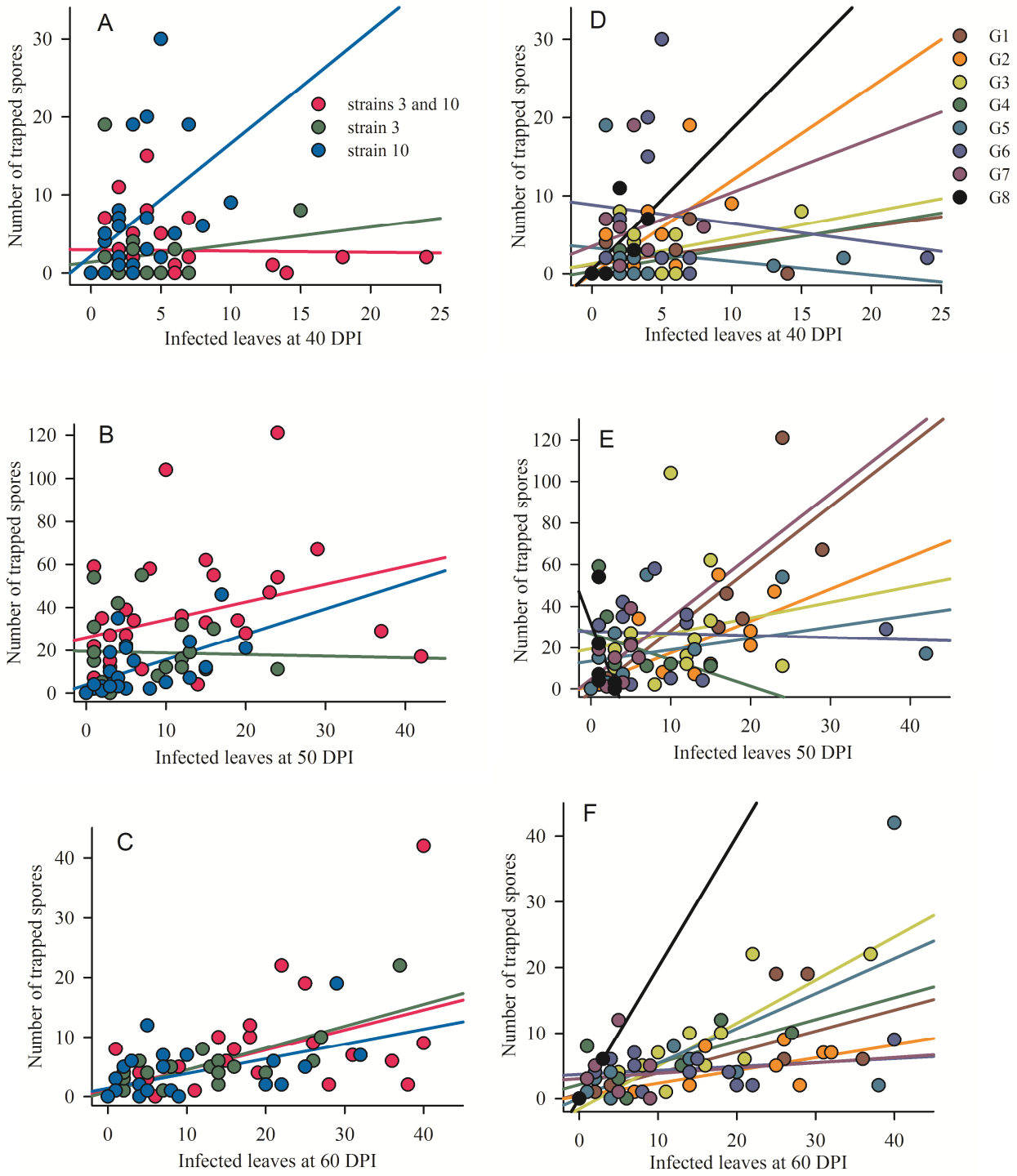
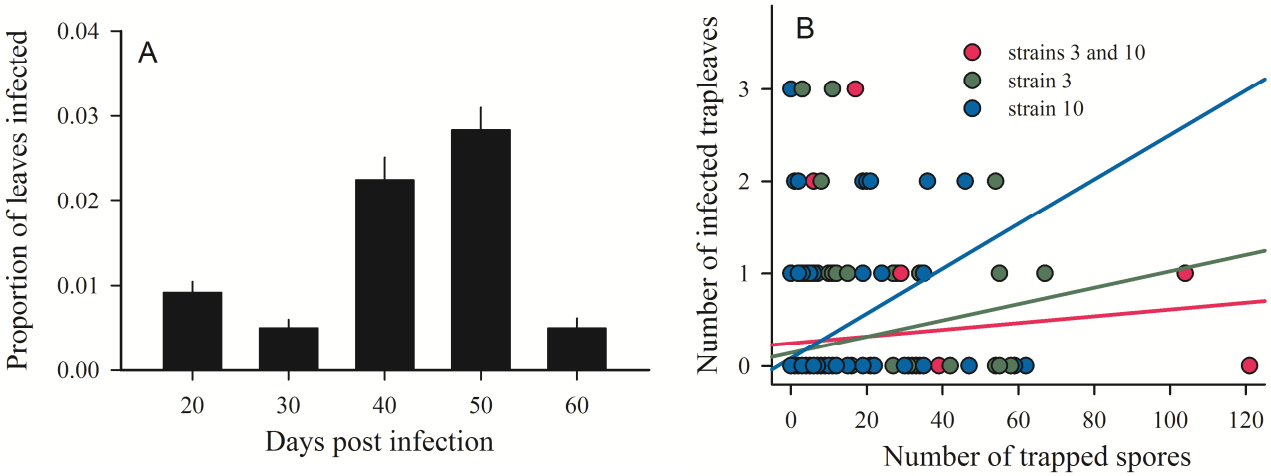

A
Number of trapped spores
Infected leaves at 40 DPI
strains 3 and 10
strain 3
strain 10
D
G1
G2
G3
G4
G5
G6
G7
G8
B
Infected leaves at 50 DPI
E
C
Infected leaves at 60 DPI
F

Figure3.


A
Proportion of leaves infected
0.04
0.03
0.02
0.01
0.00
20
30
40
50
60
Days post infection
B
Number of infected trapleaves
3
2
1
0
strains 3 and 10
strain 3
strain 10
0
20
40
60
80
100
120
Number of trapped spores